NR

PATENT COOPERATION TREAT

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 29 November 2000 (29.11.00)	
International application No. PCT/EP00/03913	**Applicant's or agent's file reference** 001164woMekk
International filing date (day/month/year) 02 May 2000 (02.05.00)	**Priority date** (day/month/year) 03 May 1999 (03.05.99)
Applicant NITSCH, Roger et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 November 2000 (09.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Claudio Borton
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

ENT COOPERATION TREATY

09/926442

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 b low.	
001164woMekk		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 03913	02/05/2000	03/05/1999
Applicant		
EVOTEC BIOSYSTEMS AG		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [X] **Unity of Invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   METHODS OF DIAGNOSING OR TREATING ALZHEIMER'S DISEASE

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

   [X] as suggested by the applicant.

   [ ] None of the figures.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25, 27-28

   Diagnosis or monitoring or treatment or prevention of Alzheimer's disease involving NGF (Nerve Growth Factor).

2. Claim : 26

   A method of screening for agents influencing the activity or level of NGF (Nerve Growth Factor) or a gene coding for NGF.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 24-25 (partially), 27-28 (partially)

Present claims 24-25 and 27-28 relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of NGF (Nerve Growth Factor) or a gene coding for NGF. Present claims 27-28 additionally relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of a further neurotrophin or a gene coding therefor.

No technical features of the agents are present in the above-mentioned claims which would lead to this desirable property, the technical features formulated so as to permit the execution of a meaningful search. No support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found for the substances which could fall within the scope of these claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. No means are present in the above-mentioned claims by which agents known in the prior art could be distinguished from novel agents. No definition of the subject matter for which protection is sought is therefore derivable from these claims (Article 6 PCT) or the description (Article 5 PCT). Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search for claims 24,25,27 and 28 has been restricted to the substances which are clearly defined and supported by the description, namely NGF (Nerve Growth Factor), optionally in combination with a further neurotrophin, in so far as they achieve the result of modulating the level of NGF / a further neurotrophin.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]P 00/03913

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DICOU E; VERMERSCH P; PENISSON-BESNIER I; DUBAS F; NERRI ERE V: "Anti-NGF autoantibodies and NGF in sera of Alzheimer patients and in normal subjects in relation to age" AUTOIMMUNITY, vol. 26, no. 3, 1997, pages 189-194, XP000852982 cited in the application the whole document --- -/--	1-11, 15-18, 22,23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 September 2000	06/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LORIGADOS L; SODERSTROM S; EBENDAL T: "Two-site enzyme immunoassay for beta NGF applied to human patient sera" JOURNAL OF NEUROSCIENCE RESEARCH, vol. 32, no. 3, July 1992 (1992-07), pages 329-339, XP000852933 cited in the application the whole document	1-11, 15-18, 22,23
X	MASSARO A R; SORANZO C; BIGON E; BATTISTON S; MORANDI A; CARNEVALE A; CALLEGARO L: "Nerve growth factor (NGF) in cerebrospinal fluid (CSF) from patients with various neurological disorders" ITALIAN JOURNAL OF NEUROLOGICAL SCIENCES, vol. 15, no. 2, March 1994 (1994-03), pages 105-108, XP000852926 cited in the application the whole document	1-11, 15-18, 22,23
X	CRUTCHER K A; SCOTT S A; LIANG S; EVERSON W V; WEINGARTNER J: "Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease" JOURNAL OF NEUROSCIENCE, vol. 13, no. 6, June 1993 (1993-06), pages 2540-2550, XP000852929 cited in the application figures 6,7	1-11, 15-18, 22,23
X	WO 91 19982 A (FIDIA SPA) 26 December 1991 (1991-12-26) cited in the application page 14, line 31, paragraph 3; examples 1,2	1-11, 15-18, 22,23
X	HOCK CHRISTOPH; HEESE KLAUS; MUELLER-SPAHN FRANZ; HULETTE CHRISTINE; ROSENBERG CARLYN; OTTEN UWE: "Decreased trkA neurotrophin receptor expression in the parietal cortex of patients with Alzheimer's disease" NEUROSCIENCE LETTERS, vol. 241, 30 January 1998 (1998-01-30), pages 151-154, XP000949445 the whole document	1-23

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SEIGER A, NORDBERG A, VON HOLST H, BACKMAN L, EBENDAL T, ALAFUZOFF I, AMBERLA K, HARTVIG P, HERLITZ A, LILJA A, ET AL: "Intracranial infusion of purified nerve growth factor to an Alzheimer patient: the first attempt of a possible future treatment strategy" BEHAVIOURAL BRAIN RESEARCH, vol. 57, no. 2, 30 November 1993 (1993-11-30), pages 255-261, XP000852665 cited in the application the whole document	24,25
X	B HOFFER, L OLSON: "Treatment strategies for neurodegenerative diseases based on trophic factors and cell transplantation techniques" JOURNAL OF NEURAL TRANSMISSION. SUPPLEMENTUM, vol. 49, 1997, pages 1-10, XP000852673 cited in the application the whole document	24,25
X	LAPCHAK P A: "NERVE GROWTH FACTOR PHARMACOLOGY: APPLICATION TO THE TREATMENT OF CHOLINERGIC NEURODEGENERATION IN ALZHEIMER'S DISEASE" EXPERIMENTAL NEUROLOGY, vol. 124, 1 January 1993 (1993-01-01), pages 16-20, XP002037880 cited in the application the whole document	1-11, 15-18, 22-25
X	SOFRONIEW, MICHAEL V.: "Nerve growth factor, ageing and Alzheimer's disease" ALZHEIMER S RESEARCH, vol. 2, no. 1-2, 1996, pages 7-13, XP000852943 cited in the application the whole document	1-11, 15-18, 22-25
X	WO 94 19461 A (CEPHALON INC) 1 September 1994 (1994-09-01) claims 1,13	24-26
A	NISHIO T, SUNOHARA N, MIZUTANI K, AKIGUCHI I, FURUKAWA S: "Nerve growth factor levels in cerebrospinal fluid are high in the inflammatory neurological disorders" CLINICA CHIMICA ACTA, vol. 275, no. 1, 6 July 1998 (1998-07-06), pages 93-98, XP000852979 cited in the application the whole document	1-11, 15-18, 22-25

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	LAPPALAINEN R; LINDHOLM D; RIIKONEN R: "Low levels of nerve growth factor in cerebrospinal fluid of children with Rett syndrome" JOURNAL OF CHILD NEUROLOGY, vol. 11, no. 4, July 1996 (1996-07), pages 296-300, XP000852954 cited in the application the whole document	1-11, 15-18, 22,23
A	WESKAMP G, OTTEN U: "An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues" JOURNAL OF NEUROCHEMISTRY, vol. 48, no. 6, June 1987 (1987-06), pages 1779-1786, XP000852664 cited in the application the whole document	1-11, 15-18, 22-25
A	MURASE, KATSUHITO; NABESHIMA, TOSHITAKA; ROBITAILLE, YVES; QUIRION, REMI; OGAWA, MICHIKO; HAYASHI, KYOZO: "NGF level is not decreased in the serum, brain-spinal fluid, hippocampus, or parietal cortex of individuals with Alzheimer's disease" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 193, no. 1, 1993, pages 198-203, XP002122252 cited in the application the whole document	1-11, 15-18, 22,23

INTERNATIONAL SEARCH REPORT

Info on patent family members

International Application No
P[redacted]EP 00/03913

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9119982 A	26-12-1991	IT 1243281 B	26-05-1994
		AU 7969091 A	07-01-1992
		CN 1058846 A	19-02-1992
		EP 0533779 A	31-03-1993
WO 9419461 A	01-09-1994	NONE	

CLAIMS

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

2. A method of monitoring progression of Alzheimer's disease in a subject, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

3. A method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of a subject; and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

4. The method according to any of claims 1 to 3, wherein an increase of said level of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

5. The method according to claim 4, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. The method according to claim 5, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

7. The method according to any of claims 1 to 6, wherein said subject is a human.

8. The method according to any of claims 1 to 7, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

9. The method according to any of claims 1 to 8, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

10. The method according to any of claims 1 to 9, wherein said subject receives a treatment prior to one or more of said sample gatherings.

11. The method according to any of claims 1 to 10, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

12. The method according to any of claims 1 to 11, further comprising:
determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;
wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

13. The method according to claim 12 wherein said neurotrophin is neurotrophin-3.

14. The method according to claim 13 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

15. A kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

16. The kit according to claim 15, wherein an increase of said level of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. The kit according to claim 16, wherein a level of nerve growth factor $\geq$ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

18. The kit according to claim 17, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to

14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

19. The kit according to any of claims 15 to 18 further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

20. The kit according to claim 19 wherein said neurotrophin is neurotrophin-3.

21. The kit according to claim 20 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

22. The kit according to any of claims 15 to 21 for use in monitoring a progression of Alzheimer's disease in a subject.

23. The kit according to any of claims 15 to 21 for use in monitoring the success or failure of a therapeutic treatment of a subject.

24. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

25. Use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

26. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

27. A composition for use as a medicament comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin and a further neurotrophin.

28. A composition according to claim 27 wherein said further neurotrophin is neurotrophin 3.

09/926442

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
001164woMe/ge		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/03913	02/05/2000	03/05/1999
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant EVOTEC BIOSYSTEMS AG et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/11/2000	31.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cuendet, P Telephone No. +49 89 2399 8690

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/03913

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17	as originally filed				

Claims, No.:

1-26	as received on	16/07/2001	with letter of	13/07/2001	

Drawings, sheets:

1/5-5/5	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 22,23.

because:

   ☒ the said international application, or the said claims Nos. 22,23 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22,23 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☒ the claims, or said claims Nos. 22,23 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

   ☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-21.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-13, 15-21
	No:	Claims	1-3, 14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1). **Preamble**

1.1. According to the all the figures, tables and examples of the present application, the present invention relates to the biochemical diagnosis of Alzheimer's disease (AD), cf. e.g. on p.11, "Testing either NGF levels or NGF and NT-3 levels with suitable cut-off criteria constitutes candidate tools for specific biochemeical diagnosis of AD".

1.2. The claims indicate/combine elements which do not belong to the concept of the biochemical diagnosis according to the figures, tables and examples of the application, cf. "prognosing AD", "monitoring the progression of AD", "evaluating a treatment for AD", "treating AD", "varied activity".

1.3. The combination "both said increase in said level and said varied activity" (cf. claims 1, 2, 14) extends beyond the application as originally filed.

1.4. Claim 14 has not been restricted to an "increase in said level", cf. option in that claim "or a level...".

2). **Point IV.**

The **subject-matter of the claims** of the present application would appear to relate to the following **2 inventions**:

First invention: method for diagnosis/monitoring/treatment/prevention of Alzheimer's disease involving nerve growth factor (NGF); cf. claims 1-21.

Second invention: method for identifying an agent influencing the activity and level of NGF and composition comprising that agent; cf. claims 24-26.

The 2 inventions are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons: to identify/use an "agent" which influences NGF is not in the context of diagnosing AD (AD is not indicated in these claims).

It is considered that the first invention is the main invention. This IPER is established for the first invention only, i.e. for claims 1-21; no opinion is given for claims 24-26 belonging to the second invention.

3). **Point III.**

3.1. No opinion is given for claims 22-23 because these claims would appear to lack support and sufficient disclosure (Art. 5 and 6 PCT); see comments made under Box I.2. of the International search report which was established for the

present application, cf. report, "Continuation of Box I.2....." (it should be noted that these comments made under Box I.2 would also apply to the claims belonging to the second invention).

3.2. The applicant is also reminded of Rule 67.1(iv) PCT; for claims 22-23 no opinion can be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4). **Point V.2.**

4.1. The detection of the levels of NGF in cerebrospinal fluid CSF/the items to detect NGF, in particular a selective reagent for NGF, were known from D12: paper by Nishio et al. or D15: Murase et al. D15 also discloses that patients with AD or VD (vascular dementia)/AD and VD have a sightly higher NGF level in brain-spinal fluid than normal controls; cf. D15, fig.2 and p.201, second paragraph. According to D5: WO-A-91/19982, p.14, last paragraph and claims 20 and 21 a skilled person would have been led to look for altered NGF levels in e.g. CSF of AD patients. Thus the present kit-claims (14-21) using such a reagent would appear to lack novelty/an inventive step in the light of D12, D15 or D5.

4.2. Method-claims 1-13 would appear to lack novelty regarding D15, cf. the slightly higher NGF levels in patients with AD or VD/AD and VD as indicated in above point 4.1. Once the applicant will have restricted the claims to a clearly definded method with the defined technical features of the invention (see in Points III above and VII and VIII below), the applicant will have to indicate why such a method would be inventive regarding the documents D15, D12, D5 or a combination thereof.

5). **Point VII.**

Unclear technical features are present in claims 1, 2, 11, 14 and 17, cf. "varied activity", "varied level", the term "reference value" defined by a desirable property, the term "one reagent" defined by a desirable property, the term "diagnosing"/ "prognosing"/"determining" defined by the result to be achieved. Furthermore, in these claims, the very large number of possibilities would appear to be in conflict

with the basic provisions of support and sufficient disclosure (should the applicant decide to proceed to the regional phase before the EPO, the applicant is reminded of the Official Journal EPO, 5/2000, pp.228-234). Furthermore, claims 1, 2 and 14 would appear to be in conflict with Art. 19.2 PCT, see point 1.3. above. Claim 3, 20 and 21 would appear to lack sufficient disclosure, see in point 1.2. above (Art. 5 PCT).

6). **Point VIII.**

Unclear technical features are present in claims 1, 2, 11, 14 and 17, cf. "varied activity", "varied level", the term "reference value" defined by a desirable property, the term "one reagent" defined by a desirable property, the term "diagnosing"/ "prognosing"/"determining" defined by the result to be achieved. Furthermore, with the very large number of possibilities which are indicated in these claims, these claims would appear to be in conflict with the basic provisions of clarity and conciseness.

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
001164woMe/ge		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/03913	02/05/2000	03/05/1999
International Patent Classification (IPC) or national classification and IPC G01N33/68		
Applicant EVOTEC BIOSYSTEMS AG et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 8 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/11/2000	31.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cuendet, P Telephone No. +49 89 2399 8690

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/03913

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-17	as originally filed				

Claims, No.:

1-26	as received on	16/07/2001	with letter of	13/07/2001	

Drawings, sheets:

1/5-5/5	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 22,23.

because:

☒ the said international application, or the said claims Nos. 22,23 relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 22,23 are so unclear that no meaningful opinion could be formed (*specify*):
see separate sheet

☒ the claims, or said claims Nos. 22,23 are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:
see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-21.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4-13, 15-21
	No:	Claims	1-3, 14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-21
Industrial applicability (IA)	Yes:	Claims	1-21
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the int rnational application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1). **Preamble**

1.1. According to the all the figures, tables and examples of the present application, the present invention relates to the biochemical diagnosis of Alzheimer's disease (AD), cf. e.g. on p.11, "Testing either NGF levels or NGF and NT-3 levels with suitable cut-off criteria constitutes candidate tools for specific biochemeical diagnosis of AD".

1.2. The claims indicate/combine elements which do not belong to the concept of the biochemical diagnosis according to the figures, tables and examples of the application, cf. "prognosing AD", "monitoring the progression of AD", "evaluating a treatment for AD", "treating AD", "varied activity".

1.3. The combination "both said increase in said level and said varied activity" (cf. claims 1, 2, 14) extends beyond the application as originally filed.

1.4. Claim 14 has not been restricted to an "increase in said level", cf. option in that claim "or a level...".

2). **Point IV.**

The **subject-matter of the claims** of the present application would appear to relate to the following **2 inventions**:

First invention: method for diagnosis/monitoring/treatment/prevention of Alzheimer's disease involving nerve growth factor (NGF); cf. claims 1-21.

Second invention: method for identifying an agent influencing the activity and level of NGF and composition comprising that agent; cf. claims 24-26.

The 2 inventions are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons: to identify/use an "agent" which influences NGF is not in the context of diagnosing AD (AD is not indicated in these claims).

It is considered that the first invention is the main invention. This IPER is established for the first invention only, i.e. for claims 1-21; no opinion is given for claims 24-26 belonging to the second invention.

3). **Point III.**

3.1. No opinion is given for claims 22-23 because these claims would appear to lack support and sufficient disclosure (Art. 5 and 6 PCT); see comments made under Box I.2. of the International search report which was established for the

present application, cf. report, "Continuation of Box I.2....." (it should be noted that these comments made under Box I.2 would also apply to the claims belonging to the second invention).

3.2. The applicant is also reminded of Rule 67.1(iv) PCT; for claims 22-23 no opinion can be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4). **Point V.2.**

4.1. The detection of the levels of NGF in cerebrospinal fluid CSF/the items to detect NGF, in particular a selective reagent for NGF, were known from D12: paper by Nishio et al. or D15: Murase et al. D15 also discloses that patients with AD or VD (vascular dementia)/AD and VD have a sightly higher NGF level in brain-spinal fluid than normal controls; cf. D15, fig.2 and p.201, second paragraph. According to D5: WO-A-91/19982, p.14, last paragraph and claims 20 and 21 a skilled person would have been led to look for altered NGF levels in e.g. CSF of AD patients. Thus the present kit-claims (14-21) using such a reagent would appear to lack novelty/an inventive step in the light of D12, D15 or D5.

4.2. Method-claims 1-13 would appear to lack novelty regarding D15, cf. the slightly higher NGF levels in patients with AD or VD/AD and VD as indicated in above point 4.1. Once the applicant will have restricted the claims to a clearly definded method with the defined technical features of the invention (see in Points III above and VII and VIII below), the applicant will have to indicate why such a method would be inventive regarding the documents D15, D12, D5 or a combination thereof.

5). **Point VII.**

Unclear technical features are present in claims 1, 2, 11, 14 and 17, cf. "varied activity", "varied level", the term "reference value" defined by a desirable property, the term "one reagent" defined by a desirable property, the term "diagnosing"/ "prognosing"/"determining" defined by the result to be achieved. Furthermore, in these claims, the very large number of possibilities would appear to be in conflict

with the basic provisions of support and sufficient disclosure (should the applicant decide to proceed to the regional phase before the EPO, the applicant is reminded of the Official Journal EPO, 5/2000, pp.228-234). Furthermore, claims 1, 2 and 14 would appear to be in conflict with Art. 19.2 PCT, see point 1.3. above. Claim 3, 20 and 21 would appear to lack sufficient disclosure, see in point 1.2. above (Art. 5 PCT).

6). **Point VIII.**

Unclear technical features are present in claims 1, 2, 11, 14 and 17, cf. "varied activity", "varied level", the term "reference value" defined by a desirable property, the term "one reagent" defined by a desirable property, the term "diagnosing"/ "prognosing"/"determining" defined by the result to be achieved. Furthermore, with the very large number of possibilities which are indicated in these claims, these claims would appear to be in conflict with the basic provisions of clarity and conciseness.

Claims (amended)

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

2. A method of monitoring progression of Alzheimer's disease in a subject, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

3. Use of the method according to claims 1 or 2 for evaluating a treatment for Alzheimer's disease.

4. The method according to any of claims 1 to 3, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

5. The method according to claim 4, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. The method according to any of claims 1 to 5, wherein said subject is a human.

7. The method according to any of claims 1 to 6, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

8. The method according to any of claims 1 to 7, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

9. The method according to any of claims 1 to 8, wherein said subject receives a treatment prior to one or more of said sample gatherings.

10. The method according to any of claims 1 to 9, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

11. The method according to any of claims 1 to 10, further comprising:

determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;

and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;

wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status

indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

12. The method according to claim 11 wherein said neurotrophin is neurotrophin-3.

13. The method according to claim 12 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

14. A kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein an increase in said level, or a varied activity, or both said increase in said level and said varied activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

15. The kit according to claim 14 wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

16. The kit according to claim 15 wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. The kit according to any of claims 14 to 16 further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status,

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

18. The kit according to claim 17 wherein said neurotrophin is neurotrophin-3.

19. The kit according to claim 18 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

20. The kit according to any of claims 14 to 19 for use in monitoring a progression of Alzheimer's disease in a subject.

21. The kit according to any of claims 14 to 19 for use in monitoring the success or failure of a therapeutic treatment of a subject.

22. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective

amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

23. Use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

24. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

25. A composition for use as a medicament comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin and a further neurotrophin.

26. A composition according to claim 25 wherein said further neurotrophin is neurotrophin-3.

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27. JULI 2000

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 18 July 2000 (18.07.00)	
Applicant's or agent's file reference 001164woMekk	**IMPORTANT NOTIFICATION**
International application No. PCT/EP00/03913	International filing date (day/month/year) 02 May 2000 (02.05.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 03 May 1999 (03.05.99)
Applicant EVOTEC BIOSYSTEMS AG et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
03 May 1999 (03.05.99)	99108722.2	EP	30 June 2000 (30.06.00)
09 Octo 1999 (09.10.99)	99120211.0	EP	30 June 2000 (30.06.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Jean-Marie McAdams

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

AvK | Sg | W | Da | Hi | HPJ | ME | TW | JH | KB

17 NOV 2000

Date of mailing (day/month/year) 09 November 2000 (09.11.00)	
Applicant's or agent's file reference 001164woMekk	**IMPORTANT NOTICE**
International application No. PCT/EP00/03913	**International filing date** (day/month/year) 02 May 2000 (02.05.00)
Priority date (day/month/year) 03 May 1999 (03.05.99)	
Applicant EVOTEC BIOSYSTEMS AG et al	

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AG,AU,DZ,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   09 November 2000 (09.11.00) under No. WO 00/67033

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/308

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year)	IMPORTANT NOTICE
09 November 2000 (09.11.00)	
Applicant's or agent's file reference	**International application No.**
001164woMekk	PCT/EP00/03913

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

PATENT COOPERATION TREATY

09/926442

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

To:

MEYERS, Hans-Wilhelm
P.O. Box 10 22 41
D-50462 Cologne
ALLEMAGNE

17. JUL 2000

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
04 July 2000 (04.07.00)	
Applicant's or agent's file reference	**International application No.**
001164woMekk	PCT/EP00/03913

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

EVOTEC BIOSYSTEMS AG (for all designated States except US)
NITSCH, Roger et al (for US)

International filing date : 02 May 2000 (02.05.00)
Priority date(s) claimed : 03 May 1999 (03.05.99)
09 October 1999 (09.10.99)

Date of receipt of the record copy by the International Bureau : 13 June 2000 (13.06.00)

List of designated Offices :

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
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The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
Jean-Marie McAdams

Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/301

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
04 July 2000 (04.07.00)	
Applicant's or agent's file reference	**International application No.**
001164woMekk	PCT/EP00/03913

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [ ] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

09/926442

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/03913

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DICOU E; VERMERSCH P; PENISSON-BESNIER I; DUBAS F; NERRI ERE V: "Anti-NGF autoantibodies and NGF in sera of Alzheimer patients and in normal subjects in relation to age" AUTOIMMUNITY, vol. 26, no. 3, 1997, pages 189-194, XP000852982 cited in the application the whole document --- -/--	1-11, 15-18, 22,23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 September 2000	06/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LORIGADOS L; SODERSTROM S; EBENDAL T: "Two-site enzyme immunoassay for beta NGF applied to human patient sera" JOURNAL OF NEUROSCIENCE RESEARCH, vol. 32, no. 3, July 1992 (1992-07), pages 329-339, XP000852933 cited in the application the whole document	1-11, 15-18, 22,23
X	MASSARO A R; SORANZO C; BIGON E; BATTISTON S; MORANDI A; CARNEVALE A; CALLEGARO L: "Nerve growth factor (NGF) in cerebrospinal fluid (CSF) from patients with various neurological disorders" ITALIAN JOURNAL OF NEUROLOGICAL SCIENCES, vol. 15, no. 2, March 1994 (1994-03), pages 105-108, XP000852926 cited in the application the whole document	1-11, 15-18, 22,23
X	CRUTCHER K A; SCOTT S A; LIANG S; EVERSON W V; WEINGARTNER J: "Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease" JOURNAL OF NEUROSCIENCE, vol. 13, no. 6, June 1993 (1993-06), pages 2540-2550, XP000852929 cited in the application figures 6,7	1-11, 15-18, 22,23
X	WO 91 19982 A (FIDIA SPA) 26 December 1991 (1991-12-26) cited in the application page 14, line 31, paragraph 3; examples 1,2	1-11, 15-18, 22,23
X	HOCK CHRISTOPH; HEESE KLAUS; MUELLER-SPAHN FRANZ; HULETTE CHRISTINE; ROSENBERG CARLYN; OTTEN UWE: "Decreased trkA neurotrophin receptor expression in the parietal cortex of patients with Alzheimer's disease" NEUROSCIENCE LETTERS, vol. 241, 30 January 1998 (1998-01-30), pages 151-154, XP000949445 the whole document	1-23

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEIGER A, NORDBERG A, VON HOLST H, BACKMAN L, EBENDAL T, ALAFUZOFF I, AMBERLA K, HARTVIG P, HERLITZ A, LILJA A, ET AL: "Intracranial infusion of purified nerve growth factor to an Alzheimer patient: the first attempt of a possible future treatment strategy" BEHAVIOURAL BRAIN RESEARCH, vol. 57, no. 2, 30 November 1993 (1993-11-30), pages 255-261, XP000852665 cited in the application the whole document	24,25
X	B HOFFER, L OLSON: "Treatment strategies for neurodegenerative diseases based on trophic factors and cell transplantation techniques" JOURNAL OF NEURAL TRANSMISSION. SUPPLEMENTUM, vol. 49, 1997, pages 1-10, XP000852673 cited in the application the whole document	24,25
X	LAPCHAK P A: "NERVE GROWTH FACTOR PHARMACOLOGY: APPLICATION TO THE TREATMENT OF CHOLINERGIC NEURODEGENERATION IN ALZHEIMER'S DISEASE" EXPERIMENTAL NEUROLOGY, vol. 124, 1 January 1993 (1993-01-01), pages 16-20, XP002037880 cited in the application the whole document	1-11, 15-18, 22-25
X	SOFRONIEW, MICHAEL V.: "Nerve growth factor, ageing and Alzheimer's disease" ALZHEIMER S RESEARCH, vol. 2, no. 1-2, 1996, pages 7-13, XP000852943 cited in the application the whole document	1-11, 15-18, 22-25
X	WO 94 19461 A (CEPHALON INC) 1 September 1994 (1994-09-01) claims 1,13	24-26
A	NISHIO T, SUNOHARA N, MIZUTANI K, AKIGUCHI I, FURUKAWA S: "Nerve growth factor levels in cerebrospinal fluid are high in the inflammatory neurological disorders" CLINICA CHIMICA ACTA, vol. 275, no. 1, 6 July 1998 (1998-07-06), pages 93-98, XP000852979 cited in the application the whole document	1-11, 15-18, 22-25

2 -/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAPPALAINEN R; LINDHOLM D; RIIKONEN R: "Low levels of nerve growth factor in cerebrospinal fluid of children with Rett syndrome" JOURNAL OF CHILD NEUROLOGY, vol. 11, no. 4, July 1996 (1996-07), pages 296-300, XP000852954 cited in the application the whole document	1-11, 15-18, 22,23
A	WESKAMP G, OTTEN U: "An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues" JOURNAL OF NEUROCHEMISTRY, vol. 48, no. 6, June 1987 (1987-06), pages 1779-1786, XP000852664 cited in the application the whole document	1-11, 15-18, 22-25
A	MURASE, KATSUHITO; NABESHIMA, TOSHITAKA; ROBITAILLE, YVES; QUIRION, REMI; OGAWA, MICHIKO; HAYASHI, KYOZO: "NGF level is not decreased in the serum, brain-spinal fluid, hippocampus, or parietal cortex of individuals with Alzheimer's disease" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 193, no. 1, 1993, pages 198-203, XP002122252 cited in the application the whole document	1-11, 15-18, 22,23

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 24-25 (partially), 27-28 (partially)

Present claims 24-25 and 27-28 relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of NGF (Nerve Growth Factor) or a gene coding for NGF. Present claims 27-28 additionally relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of a further neurotrophin or a gene coding therefor.

No technical features of the agents are present in the above-mentioned claims which would lead to this desirable property, the technical features formulated so as to permit the execution of a meaningful search. No support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found for the substances which could fall within the scope of these claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. No means are present in the above-mentioned claims by which agents known in the prior art could be distinguished from novel agents. No definition of the subject matter for which protection is sought is therefore derivable from these claims (Article 6 PCT) or the description (Article 5 PCT). Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search for claims 24,25,27 and 28 has been restricted to the substances which are clearly defined and supported by the description, namely NGF (Nerve Growth Factor), optionally in combination with a further neurotrophin, in so far as they achieve the result of modulating the level of NGF / a further neurotrophin.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25, 27-28

   Diagnosis or monitoring or treatment or prevention of Alzheimer's disease involving NGF (Nerve Growth Factor).

2. Claim : 26

   A method of screening for agents influencing the activity or level of NGF (Nerve Growth Factor) or a gene coding for NGF.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/03913

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9119982 A	26-12-1991	IT 1243281 B	26-05-1994
		AU 7969091 A	07-01-1992
		CN 1058846 A	19-02-1992
		EP 0533779 A	31-03-1993
WO 9419461 A	01-09-1994	NONE	

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
G01N 33/68, 33/53

A1

(11) International Publication Number: WO 00/67033

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/EP00/03913

(22) International Filing Date: 2 May 2000 (02.05.00)

(30) Priority Data:

99108722.2	3 May 1999 (03.05.99)	EP
99120211.0	9 October 1999 (09.10.99)	EP

(71) Applicant *(for all designated States except US)*: EVOTEC BIOSYSTEMS AG [DE/DE]; Schnackenburgallee 114, D–22525 Hamburg (DE).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: NITSCH, Roger [DE/CH]; Guggerstrasse 19, CH–8702 Zollikon (CH). HOCK, Christoph [DE/CH]; Universität Zürich, Abt. für Psychiatrische Forschung, Psychiatrische Uniklinik, Lenggstrasse 31, CH–8029 Zürich (CH). OTTEN, Uwe [CH/CH]; Universität Basel, Wissens– und Technologie–Transfer–Stelle, Petersgraben 35, CH–4003 Basel (CH).

(74) Agents: MEYERS, Hans–Wilhelm et al.; P.O. Box 10 22 41, D–50462 Cologne (DE).

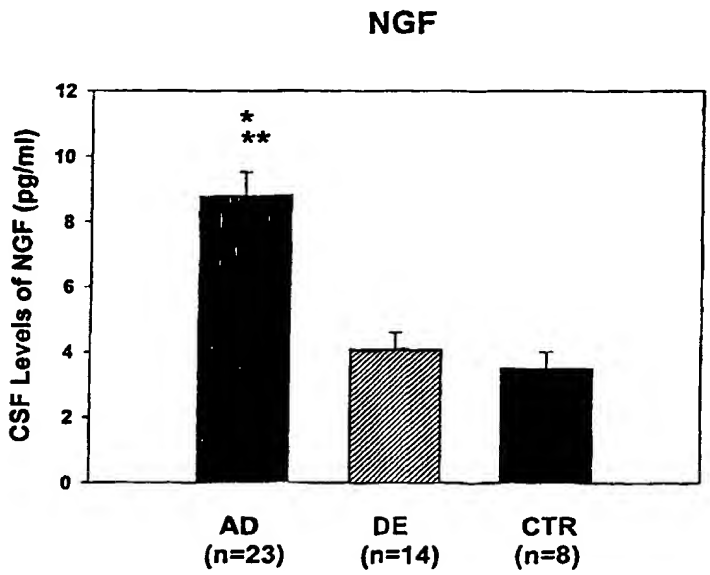
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS OF DIAGNOSING OR TREATING ALZHEIMER'S DISEASE



(57) Abstract

The invention relates to a method for diagnosing or prognosing alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising: determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken cerebrospinal fluid of said subject; and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status, wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

FOR THE PURPOSES OF INFORMATION ONLY

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DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

METHODS OF DIAGNOSING OR TREATING ALZHEIMER'S DISEASE

Alzheimer's disease (AD), first described by the Bavarian psychiatrist Alois Alzheimer in 1907, is a progressive neuropsychiatric disorder which begins with short term memory loss and proceeds to loss of cognitive functions, disorientation, impairment of judgement and reasoning and, ultimately, dementia. It is the most common form of dementia. The neuropathology is characterized by the formation in brain of amyloid plaques and neurofibrillary tangles. AD has been estimated to afflict 5 to 11 percent of the population over age 65 and as much as 47 percent of the population over age 85. Moreover, as adults, born during the population boom of the 1940's and 1950's, approach the age when AD becomes more prevalent, the control and treatment of AD will become an even more significant health care problem. Familial forms of AD are genetically heterogeneous, but most with early onset are linked to mutations in the presenilin genes *PSEN1* and *PSEN2*, as well as to mutations of the amyloid precursor gene *APP*. The majority of AD patients have no obvious family history and are classified as sporadic AD. For this late onset AD, several putative genetic risk factors have been reported. Among these the ApoE-epsilon 4 (ApoE 4) has been widely confirmed to confer increased risk for AD. Inheritance of ApoE4 and other risk factors are neither necessary nor sufficient to cause AD. In contrast to the APP- and PSEN mutations which increase the production of Aβ, the principal component of senile plaques in AD brain, the ApoE variant most likely influences Aβ accumulation by modulating clearance and degradation of the peptide.

In the search for biochemical changes in patients with neuropsychiatric and neurodegenerative disorders analysis of cerebrospinal fluid (CSF) may be a

useful method, since the CSF is continuous with the extracellular fluid of the brain. Therefore, a plurality of studies aiming at the analysis of the central nervous system (CNS) specific proteins in CSF were performed in order to find biochemical markers for neuronal and synaptic function and pathology in degenerative brain disorders.

Nerve growth factor (NGF) is one of the neurotrophic agents that promote differentiation or support the survival and functioning of some populations of neurons, influencing their effects not only on the peripheral sensory and sympathetic neurons but also on the central neurons. The pathophysiological role of NGF in the human nervous system, especially in relation to neuropsychiatric disorders, has not been fully understood yet. It is known that patients with acute multiple sclerosis (MS), traumatic brain injury or hypertensive cerebral hemorrhage show higher NGF levels in the CSF and NGF has trophic roles in regenerating axons in the CNS.

To determine the pathophysiological roles of NGF in the human CNS with special reference to neuropsychiatric disorders, levels of NGF in CSF from patients with the following neurodegenerative disorders have been examined by Nisho et al. (Clinica Chimica Acta 275, 93 - 98, 1998) using a highly sensitive two-site enzyme immunoassay:

(i) Parkinson's disease
(ii) Progressive supranuclear palsy
(iii) Sporadic olivo-ponto-cerebellar atrophy
(iv) Spinocerebellar ataxia 3 / Machado-Joseph disease
(v) Dentato-rubro-pallido-luysian atrophy

However, Nisho et al. did not examine any patients suffering from Alzheimer's disease.

Lappalainen et al. (Journal of Child Neurology 11 (4), 296 - 300, 1996) report about low levels of NGF in cerebrospinal fluid of children with Rett Syndrome.

Dicou et al. (Autoimmunity 26 (3), 189 – 194, 1997) report that no changes in anti-NGF autoantibody titers or in NGF frequency are detected in sera of AD patients, suggesting that they are not involved in the neuroimmunological mechanisms underlying AD.

Lorigados et al. (Journal of Neuroscience Research 32 (3), 329 – 339, 1992) applied a two-site enzyme immunoassay to examine NGF levels in normal human serum and serum from Alzheimer patients.

Massaro et al. (Italian Journal of Neurological Science 15 (2), 105 – 108, 1994) studied NGF in cerebrospinal fluid from patients with various neurological disorders including AD. Their study does not support the possibility that NGF is involved in the neuroimmunological mechanisms which can be expected to be linked in the inflammatory or degenerative diseases of the central and peripheral nervous system chosen in their study.

Crutcher et al. (The Journal of Neuroscience 13 (6), 2540 – 2550, 1993) used a two-site ELISA and a bioassay to detect NGF-like activity in human brain tissue. NGF-like activity was significantly elevated in the frontal and occipital cortex from patients with AD. Their results demonstrate the feasibility of detecting NGF-like activity in both fresh and postmortem human brain tissue.

The international patent application PCT/EP 91/01100 discloses a method for the qualitative and quantitative determination of a polypeptide or protein analyte present in a biological fluid or a solution. This method is exemplified by the determination of NGF.

Seiger et al. (Behavioural Brain Research 57 (2), 255 – 261, 1993) report on the clinical outcome of a first case of intracranial infusion of NGF to an AD patient. This therapeutic attempt is based on animal research showing that NGF stimulates central cholinergic neurons of the type known to be lost during the development of AD.

Hoffer et al. (Journal of Neural Transmission, Suppl. 49, 1 - 10, 1997) disclose treatment strategies based on transfer of genes, molecules, or cells to the central nervous system. Before degeneration has occurred, it may be possible to rescue "stressed" neurons, and stimulate terminal outgrowth using treatment with neurotrophic factors. Such approaches, with an emphasis on the NGF family of neurotrophins and their receptors, are reviewed.

Lapchak (Experimental Neurology 124, 16 - 20, 1993) provides in his review an overview of the importance of NGF as a neurotrophic factor for adult cholinergic neurons of the septohippocampal pathway. Information concerning the possible therapeutic use of NGF or small molecules that increase the expression of NGF to treat the cholinergic neurodegeneration that occurs in AD are provided.

Sofroniew (Alzheimer's Research 2 (1-2), 7 - 13, 1996) discloses data from pilot studies of NGF infusion into the CSF of patients with AD, peripheral administration of NGF, which suggest that achieving a method for site specific delivery of NGF in the CNS may be an important consideration in developing a treatment strategy.

Murase et al. (Biochemical and Biophysical Research Communications 193 (1), 198 - 203, 1993) disclose that NGF level is not decreased in the serum, brain-spinal fluid, hippocampus, or parietal cortex of individuals with AD.

As AD is a growing social and medical problem, there is a strong need for *ante mortem* methods of diagnosing or prognosing said disease in subjects as well as for methods of treatment.

In one aspect, the invention features a method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:

determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In a further aspect, the invention features a method of monitoring progression of Alzheimer's disease in a subject, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In still a further aspect, the invention features a method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of a subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to a said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

An increase of a level of nerve growth factor in cerebrospinal fluid from a subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject. In particular, a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject. Specifically, a level of NGF in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of AD in said subject. Said subject is preferably a human. NGF can e.g. be detected using an immunoassay, a bioassay or a binding assay (see e.g. Crutcher et al., The Journal of Neuroscience 13 (6), 2540 - 2550, 1993).

It is particularly preferred to further compare a level and/or activity of nerve growth factor with a level and/or activity of NGF in a series of samples taken from said subject over a period of time. Said subject might have received a treatment prior to one or more of said sample gatherings. Said level and/or said activity are preferably determined before and after said treatment.

In a further preferred embodiment, additionally a level, or an activity, or both said level and said activity, of a further neurotrophin, e.g. neurotrophin 3 (NT-3), is determined with the goal of diagnosing, prognosing, evaluating the risk of developing, evaluating a treatment of, or monitoring the progression of Alzheimer's disease. In particular, a level of neurotrophin 3 ≥ 15 pg/ml indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

In another aspect, the invention features a kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

In particular, an increase of said level of NGF in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of AD in said subject. In particular, a level of nerve growth factor $\geq$ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject. Specifically, a level of NGF in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of AD in said subject.

Additionally, said kit preferably further comprises at least one reagent which selectively detects neurotrophin 3 (NT-3). Combined testing of NGF and a further neurotrophin, in particular NT-3, is a valuable tool in the diagnosis, prognosis, or risk evaluation of Alzheimer's disease (see example 3 and table 1). In particular, a level of neurotrophin 3 $\geq$ 15 pg/ml indicates a diagnosis, prognosis, or increased risk of Alzheimer's disease.

In another aspect, the invention features a method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

It might be further preferred to administer to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In still another aspect, the invention features the use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

In still another aspect, the invention features a composition for use as a medicament comprising (i) a first agent which directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects, in particular reduces, an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for neurotrophin 3, a transcription product of a gene coding for neurotrophin 3, and neurotrophin 3.

In a further aspect, the invention features the use of a composition for the manufacture of a medicament for treating Alzheimer's disease, said composition comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin, and a further neurotrophin. In a preferred embodiment, said further neurotrophin is neurotrophin 3. It is preferred that said agents reduce the corresponding activity or level of NGF or NT-3, respectively.

The invention further features a method for identifying an agent that directly or indirectly affects an activity, or a level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or a level, or both said activity and level, of at least one of said substances before and after said contacting.

Figure 1 relates to example 1 and depicts that CSF levels of NGF are significantly elevated in the AD group, as compared to both the group consisting of patients with major depression (DE) as well as to the control group (CTR). Levels (pg/ml) are given in mean ± SEM. Asterisk (*, **) indicate

significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p < 0.001$; ** AD versus CTR, $p < 0.001$. NGF concentrations in CSF of the AD group amounted to 8.79 +/- 0.72 pg/ml (mean +/- SEM, range: 3.29 to 14.95, n = 23), compared to 4.07 +/- 0.50 pg/ml in the DE group (range: 2.42 to 9.54, n = 14), and 3.49 +/- 0.51 pg/ml in the CTR group (range: 0.00 to 4.64, n = 8), respectively. The alterations in patients suffering from AD may reflect disturbances in the trophic support of specific neuronal populations, such as the basal forebrain cholinergic system. There was no apparent correlation of CSF levels of NGF with ApoE genotype (or phenotype, respectively), age, duration of AD, MMS, NOSGER or MADRS scores.

Figure 2 relates to example 2 and depicts nerve growth factor levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * AD versus DE, $p = 0.002$; ** AD versus CTR, $p = 0.000$, *** DE versus CTR, $p = 0.000$. CSF levels of NGF were significantly elevated in the AD group, as compared to both the DE and the CTR group. CSF levels of NGF were also significantly elevated in the DE group, as compared to the CTR group. NGF concentrations in CSF of the AD group amounted to 8.19 +/- 0.91 pg/ml (mean +/- SEM, range: 0.00 to 23.00, n = 40), compared to 4.26 +/- 0.97 pg/ml in the DE group (range: 0.00 to 23.00, n = 22), and 1.18 +/- 0.35 pg/ml in the CTR group (range: 0.00 to 7.20, n = 32), respectively.

Figure 3 relates to example 3 and depicts neurotrophin 3 (NT-3) levels in the cerebrospinal fluid of patients with Alzheimer's disease (AD), major depression in the elderly (DE) and non-demented control subjects (CTR). CSF levels of NT-3 were determined to define a cut-off value to be used in the combined tests shown in table 1. Levels (pg/ml) are given in mean ± SEM. Asterix (*, **, ***) indicate significance ($p < 0.05$), Mann-Whitney U Test. * DE versus AD, $p = 0.005$; ** DE versus CTR, $p = 0.000$; *** AD versus CTR, $p = 0.010$. CSF levels of NT-3 were significantly elevated in the DE group, as compared to

both the AD and the CTR group. CSF levels of NT-3 were slightly, but significantly, elevated in the AD group, as compared to the CTR group. NT-3 concentrations in CSF of the DE group were 25.8 +/- 4.3 pg/ml (mean +/- SEM, range: 0.0 to 87.0, n =23), compared to 14.0 +/- 1.6 pg/ml in the AD group (range: 0.0 to 41.0, n = 39), and 10.5 +/- 1.6 pg/ml in the CTR group (range: 0.0 to 67.0, n = 63), respectively.

Table 1 relates to examples 2 and 3. This table shows the diagnostic accuracy of spinal fluid measurements of NGF and NT-3 in Alzheimer's Disease and Major Depression in the Elderly. In NGF measurements, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using a cut-off value of ≥ 4.0 pg/ml NGF (total: n = 94, AD: n = 40, DE: n = 22, CTR: n = 32). The combined NGF/NT-3 test showed a considerable specificity for the diagnosis of AD (90.1 %), using cut-off values of ≥ 4 pg/ml NGF, and < 15 pg/ml NT-3, respectively (total: n = 57, AD: n = 24, DE: n = 18, CTR: n = 15). Testing either NGF levels or NGF and NT-3 levels with suitable cut-off criteria constitutes candidate tools for specific biochemical diagnosis of AD. Using the opposite cut-off criteria, the combination test significantly separated AD patients from elderly DE patients with a specificity of 89.7 %. Therefore, another potential use of this test is the biochemical differentiation between these two frequent disorders in the elderly.

Table 2 depicts the clinical characteristics and test scores of patients with Alzheimer's disease (AD), major depression (DE) and non-demented control subjects (CTR). Nerve growth factor (NGF), neurotrophin 3 (NT-3), MMS (Mini Mental State), MADRS (Montgomery Asberg Depression Rating Scale), ApoE (apolipoprotein E), n.d. (not determined).

EXAMPLE 1

In order to achieve a differential diagnosis, the study included not only patients with AD, but also such with major depression (DE). Diagnosis of

probable AD was made according to criteria of the National Institute of Neuropsychiatric and Communicative Disorders and Stroke-Alzheimer's disease and Related Disorders Association (NINCDS-ADRDA; McKhann et al., Neurology 34, 939 – 944, 1984). Patients with major depression were diagnosed according to the ICD10 (F32.0x/1x, F33.0x/1x) and DSM-IIIR (296.20-22, 296.30-32) criteria. All patients were referred to the research ward from general practitioners, neurologists and psychiatrists for diagnostic purposes and screening for clinical trials. None of the patients was institutionalized. The group of healthy control subjects (CTR) consisted of patients that underwent lumbar puncture for orthopedic or neurologic diagnostic purposes and were shown to have normal CSF cell counts, total protein levels, and absence of signs of blood barrier dysfunction or cerebral IgG synthesis, as well as absence of any cerebral disorders.

AD, DE and CTR patients were carefully examined and received a thorough clinical work-up. Psychometric testing including the Mini Mental State (MMS; Folstein et al., J. Psychiatry Res. 12, 189 – 198, 1975), as a global screening instrument for dementia, and the Nurses' Observation Scale for Geriatric Patients (NOSGER; Spiegel et al., J. Am. Geriatr. Soc. 39(4), 339 – 347, 1991) as a functional measure of dementia severity. The patients with DE showed no cognitive disturbances in the clinical examinations and the Mini Mental State scores were within the normal range. Severity of depression was rated by using the Montgomery Asberg Depression Rating Scale (MADRS) (Montgomery et al., Br. J. Psychiatry, 134: 382 – 389, 1979). Apolipoprotein (ApoE) genotyping, or, if DNA was not available, ApoE phenotyping was included in the laboratory screening in the AD patients.

CSF was obtained for diagnostic purposes in the AD and DE patients in which no lumbar puncture had been previously done during the routine diagnostic work-up. Different CSF volumes were available for the analysis of the neurotrophin proteins. This fact explains the different sample sizes for the

individual measurements. All available CSF samples were used for the analyses.

The AD group was as follows: n = 23, 12 men, 11 women, mean age 63.9 +/- 13.2 SD years, range 39 – 86 years, MMS score: mean 18.6 +/- 5.6 SD.

The DE group was as follows: n = 14, 5 men, 9 women, mean age 68.2 +/- 13.6 SD years, range 47 – 86 years, MMS score: mean 28.1 +/- 0.9 SD.

The CTR group was as follows: n = 8, 5 men, 3 women, mean age 60.1 +/- 18.1 SD years, range 31 – 81 years.

AD and CTR patients were free of psychotropic medication. Patients with major depression were treated with various antidepressant drugs including serotonin reuptake inhibitors, reversible monoaminooxidase A inhibitors and tricyclics. Informed consent was taken from each patient and their caregivers before the investigation. The study was approved by the local ethics committee. All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983. Within one week of dementia testing, CSF was obtained by lumbar puncture. To control for possible influences of a ventriculo-lumbar gradient, lumbar punctures were done between 7.30 and 8 a. m. before breakfast while patients were still lying flat. CSF samples were frozen on dry ice immediately upon withdrawal at the bedside in 0.5 ml aliquots and stored at – 85 °C until biochemical analysis.

CSF levels of NGF were measured by an ELISA as described recently (Weskamp et al., J. Neurochem. 48, 1779 – 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim) diluted in carbonate buffer pH 9.2 over night at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room

temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D-galactopyranoside was added and plates were incubated at 4 °C over night. The reaction was stopped after 1 h at RT and the flurorescent product was measured in the microtiter wells using a fluorometer (Labsystems Fluoroskan Ascent FL) (excitation wavelength: 355 nm; emission wavelength: 460 nm). The detection limit was 1.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 %.

ApoE genotyping was performed using INNO-LiPA ApoE, Innogenetics, Belgium. ApoE phenotyping was performed according to McDowell et al. (Clin. Chem. 35(10), 2070 - 2073, 1989). The use of the ApoE phenotype synonymous with the ApoE genotype in the statistical analyses seemed to be appropriate, since ApoE genotyping compared with protein phenotyping showed conflicting results in less than 2 % (Hansen et al., Clin. Chim. Acta, 224(2), 131 - 137, 1994).

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Correlation analyses were performed by multiple regression using CSF levels of neurotrophins as well as ApoE genotype (or phenotype, respectively), age, duration of the disease in AD, MMS, NOSGER and MADRS scores. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

EXAMPLE 2

The study described in example 1 has been extended to a wider panel of patients as described below in this example 2.

Diagnosis, clinical examination and treatment of patients as well as lumbar puncture were performed as described in example 1.

For NGF measurements, 94 spinal fluid samples were examined. The AD group (n = 40) consisted of 18 men and 22 women, mean age 68.8 +/- 12.4 SD years, range 39 – 88 yr, MMS score: mean 19.3 +/- 4.6 SD. The DE group (n = 22) consisted of 8 men and 12 women, mean age 69.8 +/- 12.6 SD years, range 47 – 86 yr, MMS score: mean 27.5 +/- 2.1 SD. CTR group: n = 32, 18 men, 14 women, mean age 64.0 +/- 14.9 SD years, range 29 – 96 yr.

CSF levels of NGF were measured by an ELISA as described by Weskamp et al. (J. Neurochem. 48: 1779 – 1786, 1987). Black 96-well microplates (Nunc) were coated with monoclonal anti-β (2.5 S, 7S) NGF antibodies (Ab) (clone 27/21, Boehringer Mannheim) diluted in carbonate buffer pH 9.2 overnight at 4 °C. 120 µl of CSF and standard solutions were added and incubated for 20 hours at 4 °C. Plates were washed and incubated with anti-β (2.5 S, 7S) NGF-β-galactosidase conjugate for 2 ½ hours at room temperature (RT). Following an additional washing step, the fluorogenic substrate 4-methylumbelliferyl-β-D- galactopyranoside was added and plates were incubated at 4 °C overnight. The reaction was stopped after 1h at RT, and the fluorescent product was measured in the microtiter wells by using a fluorometer (Labsystems Fluoroskan Ascent FL) at 355 nm excitation and 460 nm emission wavelength. The detection limit was 0.5 pg/ml; the cross-reactivity with other neurotrophins at 10 ng/ml was < 2 % and the assay was linear over a range of 0.5 to 500 pg/ml.

Statistical analyses of data were performed using the Mann-Whitney U test for group comparisons. Regression analysis was complemented with analysis of variance (ANOVA) by using SPSS for Windows (version 8.0). Statistical significance was assumed at $p < 0.05$. Bonferroni correction for multiple testing was applied.

To estimate the diagnostic accuracy of the test, a) sensitivities and b) specificities, defined as follows, were calculated: a) true positives / (true

positives and false negatives), and b) true negatives / (true negatives and false positives). To estimate the probability of disease, predictive values of the tests were calculated. The positive predictive value (PPV) was defined as true positives / (true positives + false positives). The negative predictive value (NPV) was defined as true negatives / (true negatives + false negatives).

EXAMPLE 3

The purpose of this study was to check whether combined measurements of the CSF levels of NGF and neurotrophin-3 (NT-3) – which also belongs to the group of neurotrophins – improves the diagnostic accuracy of the NGF test described in example 2.

In a first step, NT-3 levels in CSF were determined to define a cut-off value to be used in the combined tests. Diagnosis, clinical examination and treatment of patients, lumbar puncture and statistical analyses were performed as described in example 2. For NT-3 measurements, 125 spinal fluid samples were examined. AD group: n = 39, 20 men, 19 women, mean age 67.2 +/- 11.5 SD years, range 39 – 86 yr, MMS score: mean 19.1 +/- 5.3 SD. DE group: n = 23, 8 men, 15 women, mean age 70.5 +/- 11.9 SD years, range 47 – 86 yr, MMS score: mean 27.2 +/- 2.5 SD. CTR group: n = 63, 35 men, 28 women, mean age 56.0 +/- 15.0 SD years, range 28 – 84 yr. NT-3 was determined by using commercially available ELISA systems (Promega, Madison, WI) according to the manufacturer's protocol. 120 µl of undiluted CSF in carbonate buffer (pH 9.7) were added to 96 well immunoplates (Nunc) at 4 °C overnight. Anti-Human-NT-3 polyclonal antibodies (pAb) were used as capture Ab. Anti-NT-3 mAb were used as reporter Ab. After incubation with a species-specific Ab (anti-rat IgG) conjugated to horseradish peroxidase (HRP) as a tertiary reactant, and washing, the solution was incubated with the chromogenic substrate TMB (3, 5, 3', 5'-tetramethylbenzidine). Absorbance

was measured at 450 nm by using a microplate reader (Dynatech MR 700). NT-3 ELISA: linear range 4.7 - 300 pg/ml; cross-reaction with other neurotrophins at 10 ng/ml < 3%; detection limit 6.0 pg/ml.

57 CSF samples were available for combined NGF/NT-3 measurements. AD group: n = 24, 13 men, 11 women, mean age 64.9 +/- 12.5 SD years, range 47 - 82 yr, MMS score: mean 18.6 +/- 5.4 SD. DE group: n = 18, 7 men, 11 women, mean age 69.5 +/- 12.7 SD years, range 47 - 84 yr, MMS score: mean 27.7 +/- 2.1 SD. CTR group: n = 15, 10 men, 5 women, mean age 59.0 +/- 15.9 SD years, range 29 - 80 yr.

CLAIMS

1. A method for diagnosing or prognosing Alzheimer's disease in a subject, or determining whether a subject is at increased risk of developing Alzheimer's disease, comprising:
   determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
   and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
   wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

2. A method of monitoring progression of Alzheimer's disease in a subject, comprising:
   determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject;
   and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
   wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

3. A method of evaluating a treatment for Alzheimer's disease, comprising:
determining a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of a subject; and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status,
wherein a varied level, or activity, or both said level and said activity, of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of said Alzheimer's disease in said subject.

4. The method according to any of claims 1 to 3, wherein an increase of said level of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

5. The method according to claim 4, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

6. The method according to claim 5, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to 14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

7. The method according to any of claims 1 to 6, wherein said subject is a human.

8. The method according to any of claims 1 to 7, wherein nerve growth factor is detected using an immunoassay, bioassay and/or binding assay.

9. The method according to any of claims 1 to 8, further comprising comparing a level and/or an activity of nerve growth factor in said sample with a level and/or an activity in a series of samples taken from said subject over a period of time.

10. The method according to any of claims 1 to 9, wherein said subject receives a treatment prior to one or more of said sample gatherings.

11. The method according to any of claims 1 to 10, wherein said level and/or activity in said samples is determined before and after said treatment of said subject.

12. The method according to any of claims 1 to 11, further comprising:
determining a level, or an activity, or both said level and said activity, of a further neurotrophin in a sample taken from cerebrospinal fluid of said subject;
and comparing said level, or said activity, or both said level and said activity, to a reference value representing a known disease or health status;
wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

13. The method according to claim 12 wherein said neurotrophin is neurotrophin-3.

14. The method according to claim 13 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

15. A kit for diagnosis, prognosis, or determination of increased risk of developing Alzheimer's disease in a subject, said kit comprising:

(a) at least one reagent which selectively detects nerve growth factor; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of nerve growth factor in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of nerve growth factor compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of nerve growth factor similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

16. The kit according to claim 15, wherein an increase of said level of nerve growth factor in said cerebrospinal fluid from said subject relative to said reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

17. The kit according to claim 16, wherein a level of nerve growth factor ≥ 4 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

18. The kit according to claim 17, wherein a level of nerve growth factor in the range from 4 pg/ml to 25 pg/ml, in particular in the range from 4 pg/ml to

14 pg/ml, in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

19. The kit according to any of claims 15 to 18 further comprising:

(a) at least one reagent which selectively detects a further neurotrophin; and

(b) instructions for diagnosing, or prognosing Alzheimer's disease, or determining increased risk of developing Alzheimer's disease by

(i) detecting a level, or an activity, or both said level and said activity, of said further neurotrophin in a sample taken from cerebrospinal fluid of said subject; and

(ii) diagnosing, or prognosing, or determining whether said subject is at increased risk of developing Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said further neurotrophin compared to a reference value representing a known health status;

or a level, or an activity, or both said level and said activity, of said further neurotrophin similar or equal to a reference value representing a known disease status

indicates a diagnosis, or prognosis, or increased risk of developing Alzheimer's disease.

20. The kit according to claim 19 wherein said neurotrophin is neurotrophin-3.

21. The kit according to claim 20 wherein a level of neurotrophin-3 $\geq$ 15 pg/ml in said cerebrospinal fluid indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

22. The kit according to any of claims 15 to 21 for use in monitoring a progression of Alzheimer's disease in a subject.

23. The kit according to any of claims 15 to 21 for use in monitoring the success or failure of a therapeutic treatment of a subject.

24. A method of treating or preventing Alzheimer's disease in a subject comprising administering to said subject in a therapeutically effective amount an agent or agents which directly or indirectly affect an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

25. Use of an agent for the manufacture of a medicament for treating Alzheimer's disease, wherein said agent directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor.

26. A method for identifying an agent that directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor, comprising the steps of:

(a) providing a sample containing at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor;

(b) contacting said sample with at least one agent;

(c) comparing an activity, or level, or both said activity and level, of at least one of said substances before and after said contacting.

27. A composition for use as a medicament comprising (i) a first agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for nerve growth factor, a transcription product of a gene coding for nerve growth factor, and nerve growth factor and (ii) a second agent which directly or indirectly affects an activity, or level, or both said activity and level, of at least one substance which is selected from the group consisting of a gene coding for a further neurotrophin, a transcription product of a gene coding for a further neurotrophin and a further neurotrophin.

28. A composition according to claim 27 wherein said further neurotrophin is neurotrophin 3.

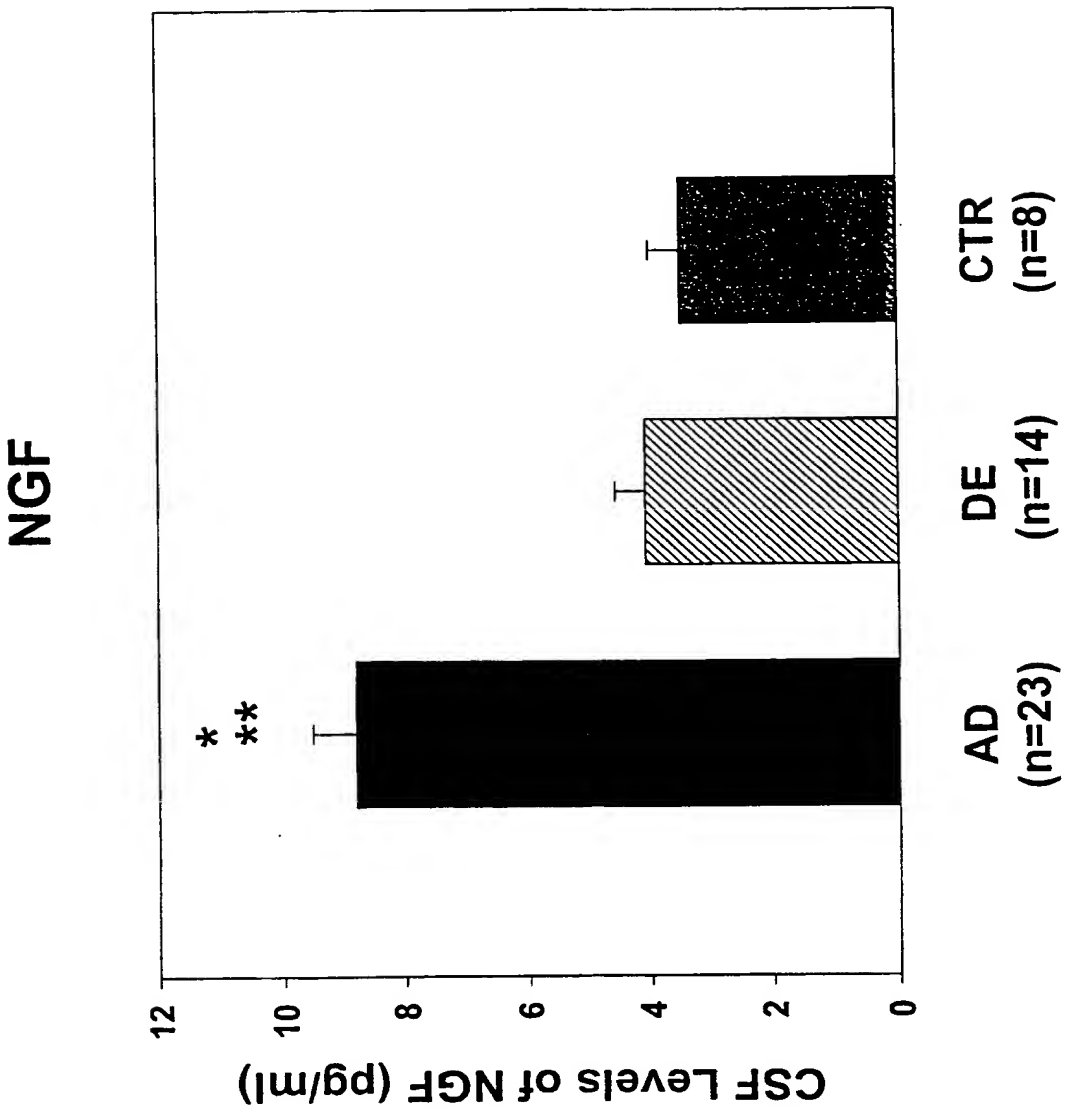

Fig. 1

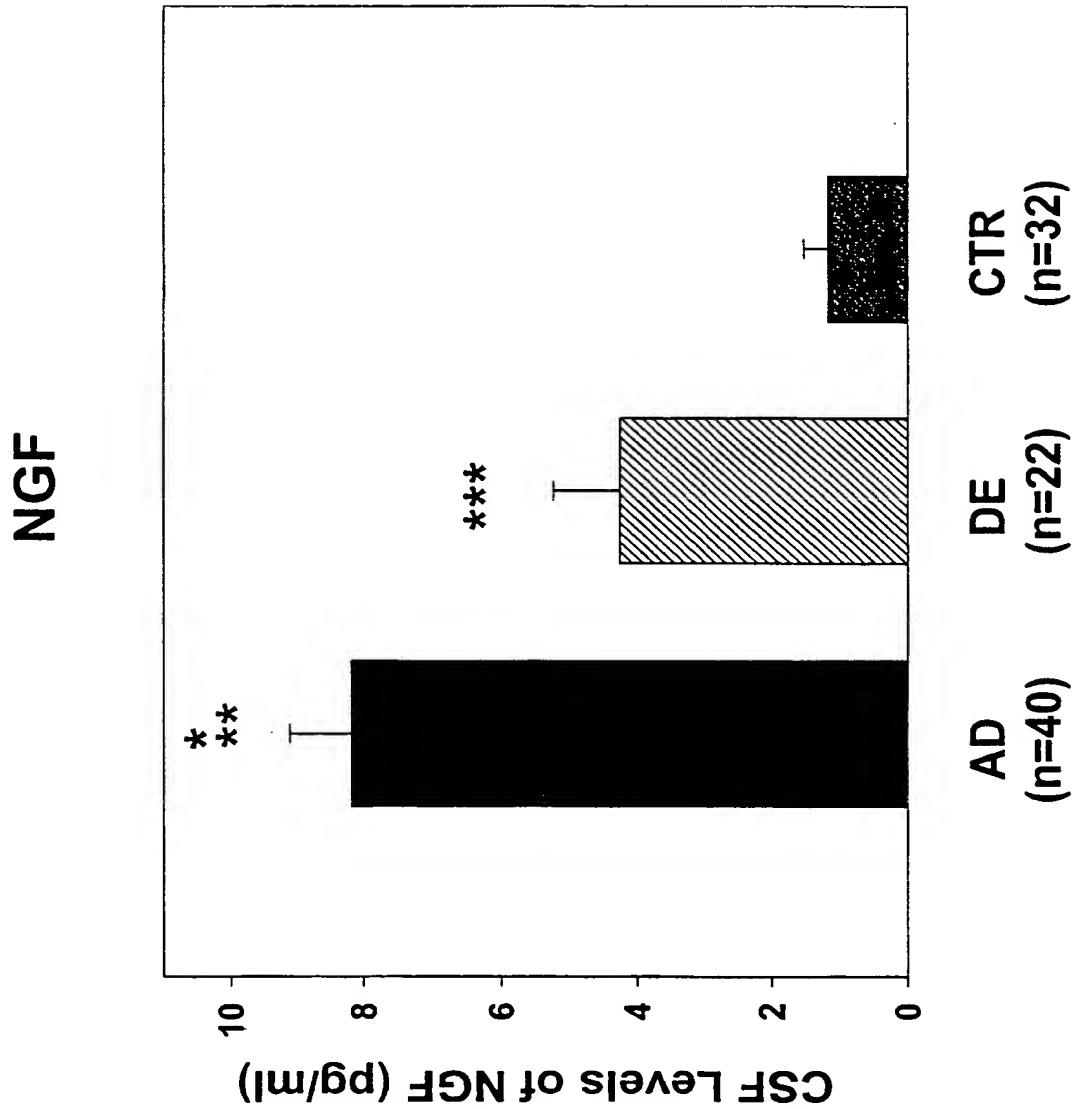

Fig. 2

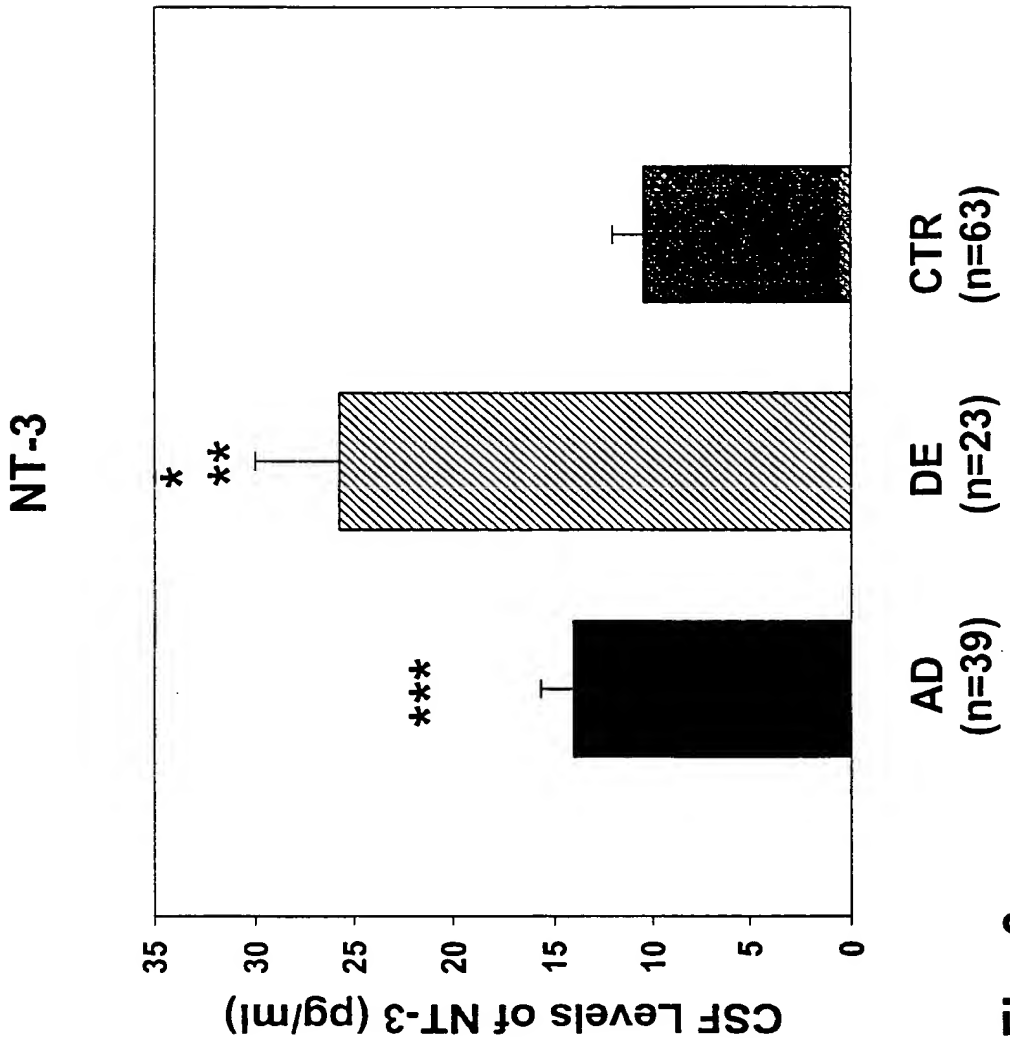
NT-3
CSF Levels of NT-3 (pg/ml)
0
5
10
15
20
25
30
35
***
**
*
AD (n=39)
DE (n=23)
CTR (n=63)

Fig. 3

Spinal Fluid Measurements of NGF and NT-3: Diagnostic Accuracy in Alzheimer's Disease (AD) and Major Depression in the Elderly (DE)

	NGF test (NGF>4 pg/ml)	**NT-3 test** (NT-3>15 pg/ml)
	Diagnosis of AD	**Diagnosis of DE**
Sensitivity	71,9%	73,9%
Specificity	79,2%	86,1%
Positive Predictive Value (PPV)	67,6%	50,0%
Negative Predictive Value (NPV)	82,4%	91,7%
	Combined NGF/NT-3 test (NGF>4 pg/ml, NT-3<15 pg/ml)	**Combined NGF/NT-3 test** (NT-3>15 pg/ml, NGF<4 pg/ml)
	Diagnosis of AD	**Diagnosis of DE**
Sensitivity	62.5%	55.5%
Specificity	90.1%	89.7%
Positive Predictive Value (PPV)	83.3%	71.4%
Negative Predictive Value (NPV)	76.9%	81.4%

Table 1

Clinical Characteristics

		AD	DE	CTR
NGF measurements	n	40 (18 m, 22 f)	21 (8 m, 13 f)	32 (18 m, 14 f)
	Age (mean±SD) yrs	68.8±12.4	69.3±12.6	64.0±14.9
	Range (yrs)	39-88	47-86	29-96
	MMS score (mean±SD)	19.3±4.6	27.6±2.1	n.d.
	MADRS score	n.d.	18.6±9.7	n.d.
	ApoE (%)	2/3 (14), 3/3 (36)	2/3 (23), 3/3 (34)	n.d.
		3/4 (43), 4/4 (7)	3/4 (31), 4/4 (12)	
NT-3 measurements	n	39 (20 m, 19 f)	n.d.	63 (35 m, 28 f)
	Age (mean±SD) yrs	67.2±11.5		56.0±15.0
	Range (yrs)	39-86		28-84
	MMS score (mean±SD)	19.1±5.3		n.d.
	MADRS score	n.d.		n.d.
	ApoE (%)	2/3 (14), 3/3 (34)		n.d.
		3/4 (45), 4/4 (7)		

Table 2

INTERNATION SEARCH REPORT

Inter. [redacted]cation No
PCT/EP 00/03913

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, EPO-Internal, WPI Data, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DICOU E; VERMERSCH P; PENISSON-BESNIER I; DUBAS F; NERRI ERE V: "Anti-NGF autoantibodies and NGF in sera of Alzheimer patients and in normal subjects in relation to age" AUTOIMMUNITY, vol. 26, no. 3, 1997, pages 189-194, XP000852982 cited in the application the whole document --- -/--	1-11, 15-18, 22,23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 September 2000	06/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	LORIGADOS L; SODERSTROM S; EBENDAL T: "Two-site enzyme immunoassay for beta NGF applied to human patient sera" JOURNAL OF NEUROSCIENCE RESEARCH, vol. 32, no. 3, July 1992 (1992-07), pages 329-339, XP000852933 cited in the application the whole document	1-11, 15-18, 22,23
X	MASSARO A R; SORANZO C; BIGON E; BATTISTON S; MORANDI A; CARNEVALE A; CALLEGARO L: "Nerve growth factor (NGF) in cerebrospinal fluid (CSF) from patients with various neurological disorders" ITALIAN JOURNAL OF NEUROLOGICAL SCIENCES, vol. 15, no. 2, March 1994 (1994-03), pages 105-108, XP000852926 cited in the application the whole document	1-11, 15-18, 22,23
X	CRUTCHER K A; SCOTT S A; LIANG S; EVERSON W V; WEINGARTNER J: "Detection of NGF-like activity in human brain tissue: increased levels in Alzheimer's disease" JOURNAL OF NEUROSCIENCE, vol. 13, no. 6, June 1993 (1993-06), pages 2540-2550, XP000852929 cited in the application figures 6,7	1-11, 15-18, 22,23
X	WO 91 19982 A (FIDIA SPA) 26 December 1991 (1991-12-26) cited in the application page 14, line 31, paragraph 3; examples 1,2	1-11, 15-18, 22,23
X	HOCK CHRISTOPH; HEESE KLAUS; MUELLER-SPAHN FRANZ; HULETTE CHRISTINE; ROSENBERG CARLYN; OTTEN UWE: "Decreased trkA neurotrophin receptor expression in the parietal cortex of patients with Alzheimer's disease" NEUROSCIENCE LETTERS, vol. 241, 30 January 1998 (1998-01-30), pages 151-154, XP000949445 the whole document	1-23

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SEIGER A, NORDBERG A, VON HOLST H, BACKMAN L, EBENDAL T, ALAFUZOFF I, AMBERLA K, HARTVIG P, HERLITZ A, LILJA A, ET AL: "Intracranial infusion of purified nerve growth factor to an Alzheimer patient: the first attempt of a possible future treatment strategy" BEHAVIOURAL BRAIN RESEARCH, vol. 57, no. 2, 30 November 1993 (1993-11-30), pages 255-261, XP000852665 cited in the application the whole document	24,25
X	B HOFFER, L OLSON: "Treatment strategies for neurodegenerative diseases based on trophic factors and cell transplantation techniques" JOURNAL OF NEURAL TRANSMISSION. SUPPLEMENTUM, vol. 49, 1997, pages 1-10, XP000852673 cited in the application the whole document	24,25
X	LAPCHAK P A: "NERVE GROWTH FACTOR PHARMACOLOGY: APPLICATION TO THE TREATMENT OF CHOLINERGIC NEURODEGENERATION IN ALZHEIMER'S DISEASE" EXPERIMENTAL NEUROLOGY, vol. 124, 1 January 1993 (1993-01-01), pages 16-20, XP002037880 cited in the application the whole document	1-11, 15-18, 22-25
X	SOFRONIEW, MICHAEL V.: "Nerve growth factor, ageing and Alzheimer's disease" ALZHEIMER S RESEARCH, vol. 2, no. 1-2, 1996, pages 7-13, XP000852943 cited in the application the whole document	1-11, 15-18, 22-25
X	WO 94 19461 A (CEPHALON INC) 1 September 1994 (1994-09-01) claims 1,13	24-26
A	NISHIO T, SUNOHARA N, MIZUTANI K, AKIGUCHI I, FURUKAWA S: "Nerve growth factor levels in cerebrospinal fluid are high in the inflammatory neurological disorders" CLINICA CHIMICA ACTA, vol. 275, no. 1, 6 July 1998 (1998-07-06), pages 93-98, XP000852979 cited in the application the whole document	1-11, 15-18, 22-25

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	LAPPALAINEN R; LINDHOLM D; RIIKONEN R: "Low levels of nerve growth factor in cerebrospinal fluid of children with Rett syndrome" JOURNAL OF CHILD NEUROLOGY, vol. 11, no. 4, July 1996 (1996-07), pages 296-300, XP000852954 cited in the application the whole document ---	1-11, 15-18, 22,23
A	WESKAMP G, OTTEN U: "An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues" JOURNAL OF NEUROCHEMISTRY, vol. 48, no. 6, June 1987 (1987-06), pages 1779-1786, XP000852664 cited in the application the whole document ---	1-11, 15-18, 22-25
A	MURASE, KATSUHITO; NABESHIMA, TOSHITAKA; ROBITAILLE, YVES; QUIRION, REMI; OGAWA, MICHIKO; HAYASHI, KYOZO: "NGF level is not decreased in the serum, brain-spinal fluid, hippocampus, or parietal cortex of individuals with Alzheimer's disease" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, vol. 193, no. 1, 1993, pages 198-203, XP002122252 cited in the application the whole document -----	1-11, 15-18, 22,23

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 24-25 (partially), 27-28 (partially)

Present claims 24-25 and 27-28 relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of NGF (Nerve Growth Factor) or a gene coding for NGF. Present claims 27-28 additionally relate to an agent defined by reference to a desirable property, namely its ability to affect the activity and/or level of a further neurotrophin or a gene coding therefor.

No technical features of the agents are present in the above-mentioned claims which would lead to this desirable property, the technical features formulated so as to permit the execution of a meaningful search. No support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found for the substances which could fall within the scope of these claims. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. No means are present in the above-mentioned claims by which agents known in the prior art could be distinguished from novel agents. No definition of the subject matter for which protection is sought is therefore derivable from these claims (Article 6 PCT) or the description (Article 5 PCT). Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

Consequently, the search for claims 24,25,27 and 28 has been restricted to the substances which are clearly defined and supported by the description, namely NGF (Nerve Growth Factor), optionally in combination with a further neurotrophin, in so far as they achieve the result of modulating the level of NGF / a further neurotrophin.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-25, 27-28

   Diagnosis or monitoring or treatment or prevention of Alzheimer's disease involving NGF (Nerve Growth Factor).

2. Claim : 26

   A method of screening for agents influencing the activity or level of NGF (Nerve Growth Factor) or a gene coding for NGF.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. [redacted] cation No
PCT/EP 00/03913

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9119982 A	26-12-1991	IT 1243281 B	26-05-1994
		AU 7969091 A	07-01-1992
		CN 1058846 A	19-02-1992
		EP 0533779 A	31-03-1993
WO 9419461 A	01-09-1994	NONE	